THE SOURCES AND ACTIVITIES OF TWO CHROMATOPHOROTROPIC HORMONES IN CRABS OF THE GENUS SESARMA. I. EXPERIMENTAL ANALYSES [1]

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As is well known (Brown, 1944; Hanström, 1947, etc.), the significance of the sinus gland as the principal source of chromatophorotropic hormone or hormones has been well substantiated by investigations, done with a large number of species in a taxonomically wide range of crustaceans since the first description of this incretory tissue by Hanström (1933). On the other hand there is a growing knowledge of the possible presence of certain hormone sources outside the sinus gland which is owed chiefly to recent continued efforts by Brown and his associates (1940–1947). They have demonstrated hormone production within the central nervous organs first in Crago, and are extending the research to other kinds of crustaceans. Thus, in the light of present information, we may infer that chromatophorotropic hormones that are directly concerned with the physiological coordination of pigmentary effector systems of crustaceans are roughly classified into two broad categories, *viz.*, the one originating in the sinus gland and the other in the nervous tissues.

Nevertheless, we are as yet only fragmentarily informed of details in connection with the actual number of hormones belonging to these respective categories, the essential activity of each of the hormones, the mode of interaction among them, etc., which is still keeping us from postulating certain generalizations with respect to the endocrine mechanism for the control of crustacean chromatophores.

Having been engaged in physiological as well as histological analyses of endocrine organizations in crabs of the genus Sesarma, with special reference to the production of chromatophorotropic hormones, I obtained some results which may furnish information concerning some of the pending problems just mentioned.

Materials and Methods

The animals used in the physiological analysis of the endocrine control of chromatophores were three representatives of the genus Sesarma, *viz.*, *S. intermedia*, *S. haematocheir* and *S. dehaani*, the most familiar brackish-water crabs in Japan. Adults of the respective species weighing ca. 30 g. (males) or ca. 25 g. (females) collected in the field were kept alive in the laboratory until required for experimentation.

For the assay of chromatophorotropic hormones present in the crabs, tissues and organs were dissected out from individuals showing no sign of ecdysis. In

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order to reject specimens in the molting phase, the early indication of the phase at the epipodite of the first maxilliped (cf. Drach, 1939) was resorted to. Isolated tissues and organs were separately ground with quartz sand to be extracted twice with distilled water. The insoluble fraction was centrifuged off, and the supernatant fluid was sealed in a glass tube and dipped into boiling water for 10 minutes. Such a preparation could be preserved for weeks and months without significant loss of hormone activity. When needed for the assay, the samples were evaporated *in vacuo* to be re-extracted with physiological saline. The final extracts were freed by filtration from coagula formed in the process of previous heating. Concentrations of extracts were expressed in terms of the number or weight of the respective tissues or organs theoretically contained in the administered doses, which were designated as T.E. (tissue equivalents). The chromatophorotropic activity of the extracts was tested chiefly upon the chromatophores of juvenile forms of *Sesarma haemato-*

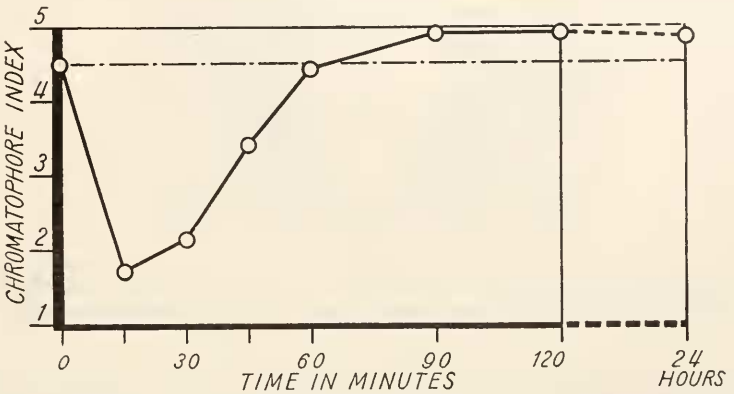

FIGURE 1. Curve showing time relation of pigmentary response of black chromatophores of juvenile *S. haematocheir* following amputation of both eyestalks.

cheir with carapace dimensions less than 20 mm. × 18 mm. Such individuals, in spite of the marked sluggishness in the chromatic display of adult forms of the three species of Sesarma, show a high chromatic activity, changing coloration from creamy yellow to chestnut brown uniformly over the whole dorsal aspect of the body including the walking legs. Background responses are lacking like in other color-changing crabs, and, under laboratory conditions during the day, the immature crabs exhibit darkening that is considerably subject to fluctuations of environmental factors such as the intensity of light, moisture and heat.

Upon bilateral amputation of the eyestalks, remarkable blanching of short duration takes place, which is followed by a process of re-darkening, and the eyestalkless animal eventually attains maximum darkness of body shade that is regarded to be the equilibrium state of pigmentary activity in the day, characteristic of the eyestalkless condition. Mainly responsible for such macroscopic changes of coloration are the brownish black chromatophores (Fig. 1), but the animal is provided with additional chromatophores, *viz.*, the red, the vermilion and the white cells, which, though limited in the chromatic effects, are also significant for the pigmentary behavior.

In the equilibrium state of the pigmentary activity of the eyestalkless animal, the black chromatophores are seen in a state of maximum pigment dispersion, the red and the vermilion cells together are in states of approximately semi-dispersion, and the white ones are in the state of nearly maximum concentration. Such states of the chromatophores were used *in vivo* and *in vitro* as test objects for the assay of the chromatophorotropic effectiveness of the extracts prepared from adult Sesarma.

Tissue or organ extracts re-extracted with known amounts of van Harreveld's solution buffered to pH 7.5 (mean pH value of the body fluid of Sesarma in the intermolt phase) were administered to the test animals that had been eyestalkless for at least two days, by injection into the thoracic musculature through the arthrodial membrane at the basal articulation of the second walking leg. Five one-hundredths cc. was injected per individual at a time; a lot of 15 test animals was employed for one assay.

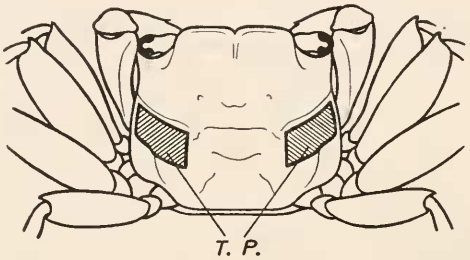

FIGURE 2. Dorsal view of juvenile *S. haematocheir*. Areas hatched (T. P.) at mesobranchial regions of dorsal carapace used as test pieces for supravital experimentation.

Extracts were tested also upon the chromatophores in isolated fragments of carapace, obtained from the mesobranchial region of the eyestalkless juvenile *S. haematocheir* (Fig. 2). As was already remarked by Brown and Meglitsch (1940), chromatophores surviving in isolated carapace fragments usually show a considerably lowered reactivity, and significant pigmentary displays are often brought about by only those distributed at the periphery of each fragment. In the present experiments, however, experience taught that frequently repeated rinsing with van Harre-

TABLE I

Size and density of four types of chromatophores in mesobranchial region of juvenile S. haematocheir of carapace dimensions of 16 mm. × 14 mm. Measurements upon eight individuals

Chromatophore / Size and Density	Black	Red	Vermilion	White
Optical Diameter at Maximum Pigment Concentration	33μ–55μ commonly ca. 38μ	11μ–33μ commonly ca. 22μ	11μ–22μ commonly ca. 17μ	15μ–33μ commonly ca. 22μ
Number in 1 mm.² of Carapace Area	87–165	6–28	74–174	96–252
Mean ± σ	120.4 ± 21.1	18.6 ± 6.9	117.9 ± 29.7	166.0 ± 54.8

veld's solution of carapace fragments directly following removal from the body was fairly good for maintaining the reactivity of chromatophores, so that use was made of carapace fragments washed for one hour in the solution that was renewed every 15 minutes. Such a treatment did not seriously affect the initial pigmentary conditions of chromatophores in the eyestalkless animal; responsiveness of the latter was preserved for an additional 10 hours under 25°C. In the assay of the extracts, 10 pieces of carapace fragments of 1 mm. × 2 mm. obtained from 10 different eyestalkless individuals were immersed in 3 cc. of the respective extract. The useful idea of T.E. was adopted too for the expression of concentrations of extracts.

TABLE II

Table summarizing chromatophorotropic activities of extracts from adult Sesarma as tested by injection upon Paratya, Ocypoda and Megaligia

Test Animal		*Paratya* Eyestalkless			*Ocypoda* Eyestalkless				*Megaligia* "Blinded"	*Megaligia* "White-adapted"
Chromatophore		Red	Yellow	White	Black	Red	Orange	White	Black	
Initial Pigmentary State		D	D	S–D	C	D	D	S–D	D	C
Effect of Injection of Extract	Sinus Gland	C	C	D?	D?	C	C	D	C?	D
	Eyestalk excluding Sinus Gland	C	C	C	D?	C	C	D		
	Optic Ganglia	C	C	D?					—	D?
	Medulla terminalis	C	C	C	D?	C	C	D	C?	D
	Brain	C	C	C	D?	C	C	D	C?	D
	Commissural Ganglion	C	C	D?					—	D?
	Thoracic Ganglion	C	C	D?	D?	C	C	D	C?	D

Notations: C, pigment concentration; D, pigment dispersion; S–D, semi-dispersion of pigment; ?, indefinite pigmentary response; —, no visible pigmentary response.

Extracts of Sesarma were further tested upon the chromatophores of *Paratya compressa,* a fresh-water shrimp, *Ocypoda stimpsoni,* a sea-shore crab, and *Megaligia exotica,* a sea-shore isopod, which all show remarkable color change. Concerning the physiology of color change of Paratya and Megaligia, papers by Nagano (1943) and Enami (1941) are to be referred to. For the present purpose, eyestalkless Paratya, eyestalkless Ocypoda, and "blinded" (eyes covered with a mixture of Sudan Black and balsam) and "white-adapted" (eyes covered at the dorsal aspect with the opaque mixture, and the animal kept in white container under dim light) Megaligia were employed. Equilibrium states of chromatophores in each of these test animals are summarized in the upper column of Table II. Van Harreveld's solution was employed as the physiological solution for Paratya, and Herbst's arti-

ficial sea-water for Ocypoda and Megaligia. The dose of injection was 0.01 cc. for Paratya, 0.05 cc. for Ocypoda and 0.02 cc. for Megaligia. Fifteen individuals of each species composed one lot of test animals.

In all these experiments, chromatophore responses *in vivo* or *in vitro* were observed at intervals of 15 minutes following administration of extracts of Sesarma, and were recorded by means of the conventional chromatophore indices. For the application of the indices to actual pigmentary states of chromatophores in a given area of the skin, the arbitrary method proposed by Hogben and Slome (1931) was adopted. Indices obtained with a lot of test objects at a given time were averaged to be plotted for a graph representing the time relation of chromatophore responses. This indicated the effectiveness of a given extract.

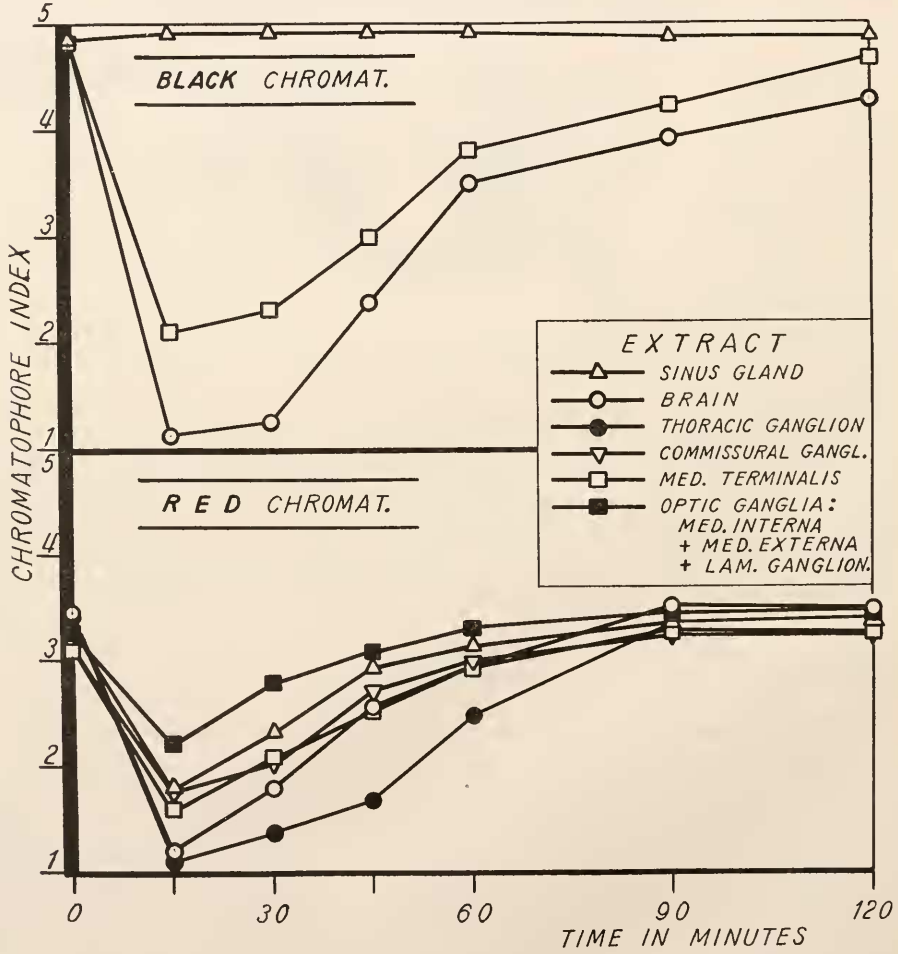

FIGURE 3. Curves showing time relations of responses of black and red chromatophores of eyestalkless *S. haematocheir*, juv. to injection of active extracts from adult *S. dehaani*. Concentration was 0.5 T.E. per 0.05 cc. in respective extracts.

Experiments and Results

Because it was concluded that in the three species of Sesarma employed the endocrine system is alike, for the sake of simplification the following account is generalized and covers all species used.

Injection Experiments upon S. haematocheir, juv.[2]

The result of injection experiments with tissue and organ extracts from adult forms of Sesarma was that only the extracts of sinus gland and nervous organs were effective in inducing definite chromatophore responses in the eyestalkless *S. haematocheir,* juv. Others, such as the extracts of stomach, intestine, hepatopancreas, heart, male genitalia, gill, muscle and hypodermis together with underlying connective tissue, were not responsible for any definite chromatophore reaction, neither was the control injection of plain van Harreveld's solution.

From the point of view of qualitative effects (cf. Fig. 3), the activities of the extracts might be arranged according to the following classification:

a) Extracts to which the red and the vermilion chromatophores were susceptible. Response: correlated pigment concentration in both chromatophores. Extracts: sinus gland, Lamina ganglionaris, Medulla externa, Medulla interna, Medulla terminalis, brain, commissural ganglion, thoracic ganglion.
b) Extracts to which the black and the white chromatophores were susceptible. Responses: pigment concentration in black chromatophores, being correlated with pigment dispersion in the white ones. Extracts: brain, Medulla terminalis.

In repeated trials of dilution experiments, the ineffectiveness of the sinus gland extract for the black and the white chromatophores was proved over a concentration range reaching as high as 5 T.E. per 0.05 cc., while a more or less significant effect was detected for the red and the vermilion cells even in a concentration as low as 0.001 T.E. per 0.05 cc. Extracts of all the principal nervous organs including brain and Medulla terminalis were effective for the red and vermilion chromatophores in various degrees, those of larger ganglia, such as thoracic ganglion, brain and Medulla terminalis, being even more effective than the sinus gland extract in the same T.E. The black and white chromatophores were affected only by extracts of brain and Medulla terminalis, the former extract exceeding the latter in effectiveness, acting even in a concentration of 0.0015 T.E. per 0.05 cc. Figure 4 shows the relation between the concentration of the brain extract and the magnitude of response of the black chromatophores, measured from time relation curves obtained by a series of dilution experiments. As is shown, the magnitude of effectiveness was exponentially proportional to the concentration of the extract, the relation between them being significantly sensitive only over a restricted range of lower concentrations.

Experiments upon Isolated Carapace Fragments of S. haematocheir, juv.

From the experiments with isolated carapace fragments obtained from eyestalkless *S. haematocheir,* juv., the qualitative activities of the active extracts of adult Sesarma were again ascertained. Observations with the carapace fragments turned

[2] Abbreviation of juvenile form.

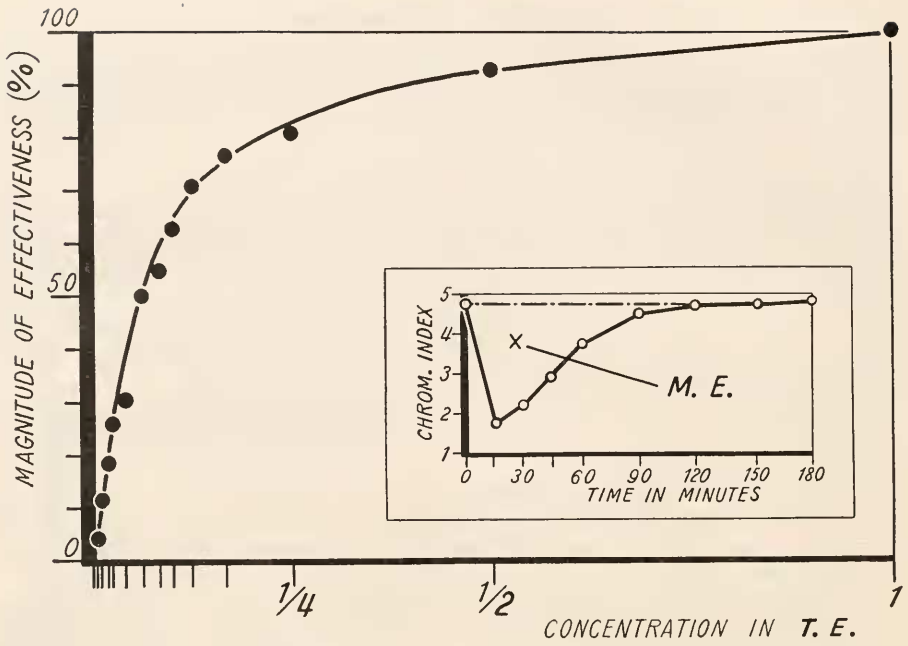

FIGURE 4. Curve showing relationship between percentage of magnitude of effectiveness and concentration of brain extract from adult *S. dehaani*. Figure inserted indicates method of estimation of magnitude of effectiveness: Area enclosed by time relation curve of response of black chromatophores and a horizontal line representing initial pigmentary state of the chromatophores was measured and designated to be magnitude of effectiveness (M.E.) of given extract. M.E. of 1 T.E. per 0.05 cc. was taken as standard value, against which percentile ratio of M.E. of respective concentration was calculated.

inside out facilitated detailed examination of the behavior of the white chromatophores, which are located in the deepest layer of the skin and are masked in various degrees when viewed from the outer surface of the carapace. The correlated responses of the cells with the black ones under the influence of the extracts of brain and Medulla terminalis were clearly demonstrated in the course of the experiments (Fig. 5).

Hormone Production by Nervous Organs

The foregoing results indicated that a kind of chromatophorotropic hormone, or a group of hormones, which, with respect to its qualitative effect, appeared to be identical with the one included in the sinus gland (sinus gland hormone), was almost universally distributed in the central nervous system of adult Sesarma. The question arose whether such a kind of hormone was originating essentially in the nervous tissue or whether it was derived from the sinus gland and stored in the former.

The question seemed to be answered in part by the fact that the effectiveness of extracts of some ganglionic tissues exceeded that of the sinus gland extract, which fact might suggest that the hormone under consideration was more concentrated in certain ganglia than in the sinus gland itself. However, definite proof of the

origin of the hormone in the nervous tissues themselves was obtained from an experiment, designed for the assay of the activity of the brain extract prepared from a lot of adult Sesarma that had been kept in the eyestalkless state for 30 days. Since the donor of the extract had been without the sinus gland, the brain extract might be reasonably concluded to be free from possible contamination of the sinus-gland hormone.

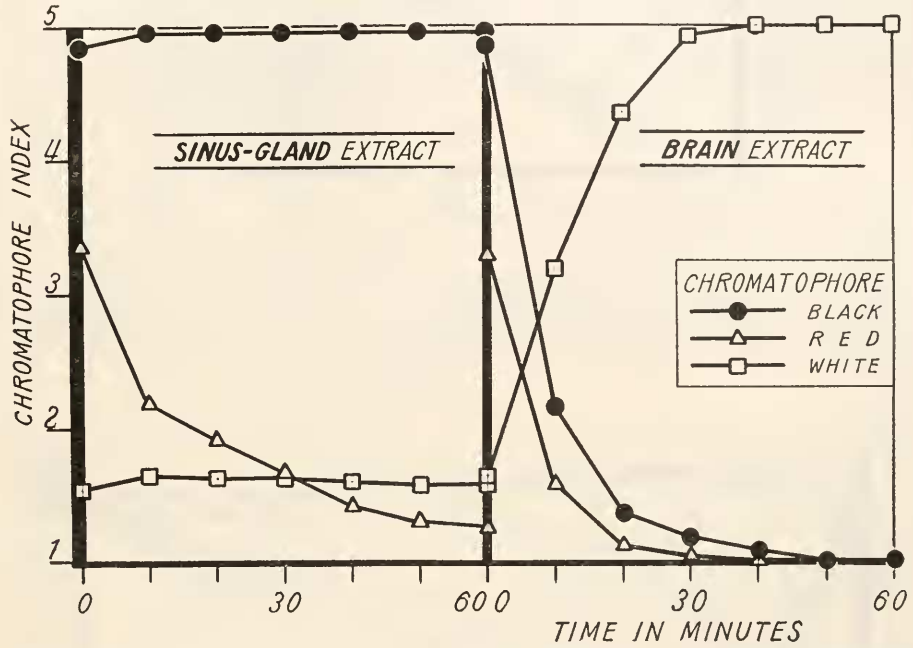

FIGURE 5. Curves showing time relations of responses of black, red and white chromatophores from eyestalkless *S. haematocheir,* juv., kept *in vitro,* to application of active extracts from adult *S. intermedia.* Concentration was 3 T.E. per 3 cc. in respective extracts.

As is shown in Figure 6, the effectiveness of the extract for the red and the vermilion chromatophores was almost comparable to that of the extract prepared from the normal animals. The same was the case with the effectiveness for the black and the white chromatophores, an observation which indicated the possible independence of the mechanism for the production of the respective hormones within the brain.

Hormone Production in Juvenile Forms of Sesarma

Injection experiments as well as experiments upon isolated carapace fragments were carried out with extracts prepared from juvenile forms of the three species of Sesarma, using the chromatophores of *S. haematocheir,* juv. as test objects. The result was that the chromatophorotropic principle known to be present in the nervous system of adult crabs was also found in juvenile forms. As to the distribution of the hormone affecting the black and the white chromatophores, it ap-

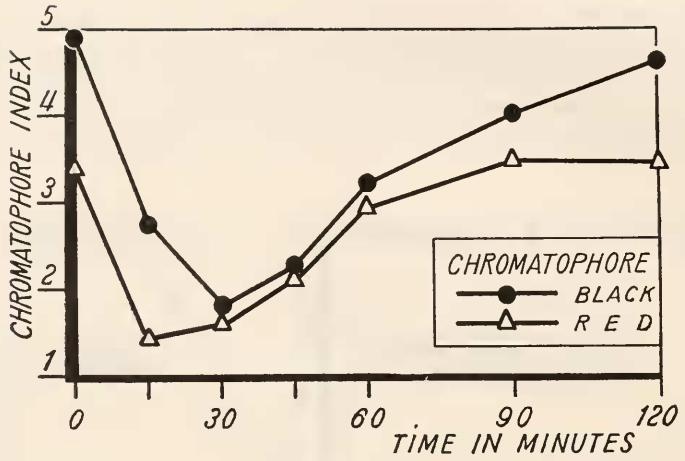

FIGURE 6. Curves showing time relations of responses of black and red chromatophores of eyestalkless *S. haematocheir*, juv. to injection of brain extract from adult *S. dehaani* that had been kept in eyestalkless state for 30 days. Concentration of extract was 0.5 T.E. per 0.05 cc.

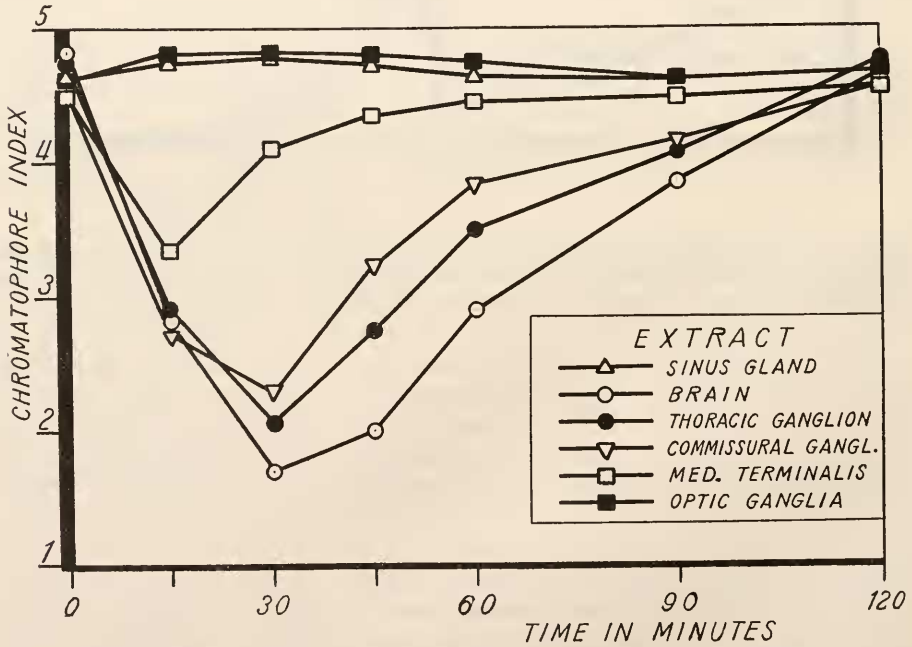

FIGURE 7. Curves showing time relations of responses of black chromatophores of eyestalkless *S. haematocheir*, juv. to injection of active extracts from juvenile *S. dehaani*. Concentration was 1 T.E. per 0.05 cc. in respective extracts.

pears to be distributed over a larger area of the nervous system in juvenile specimens than in adults, *i.e.*, in young forms not only the brain and the Medulla terminalis, but also the thoracic and commissural ganglia rendered active extracts (Fig. 7). The order of effectiveness of these extracts for the black chromatophores in the same T.E. was: brain > thoracic ganglion > commissural ganglion ≫ Medulla terminalis.

The observation that a certain incretory mechanism for the production of a kind of hormone, responsible for pigment concentration in the black chromatophores, was detected also in juvenile forms of Sesarma, suggested an explanation for the underlying mechanism of the blanching response occurring upon amputation of the eyestalks in *S. haematocheir*, juv. (cf. Fig. 1, and the account in MATERIALS AND METHODS).

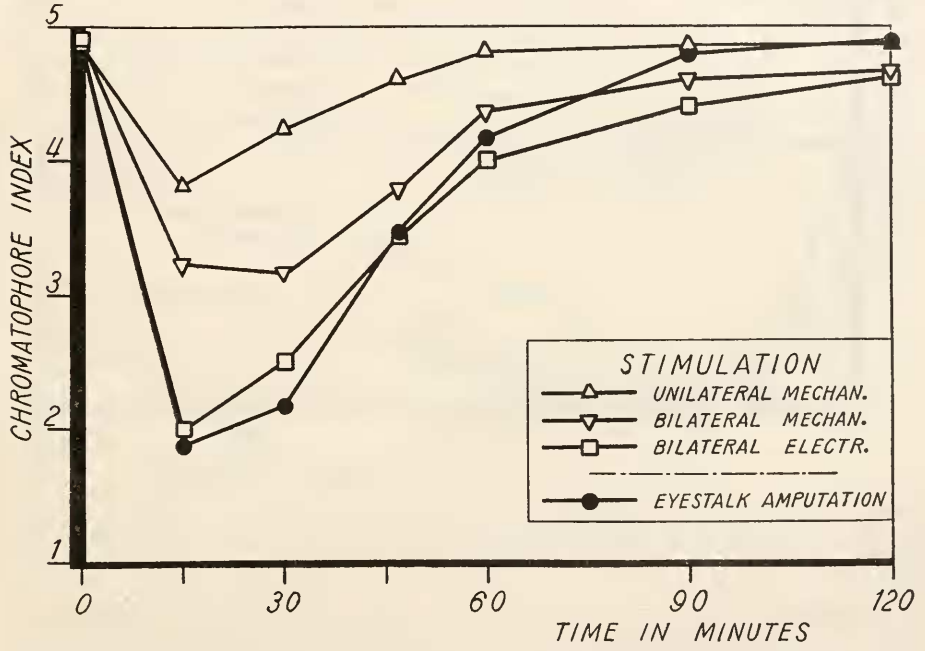

FIGURE 8. Curves showing time relations of responses of black chromatophores of eyestalkless *S. haematocheir*, juv. subjected to stimulation of stub of eyestalk.

A similar temporary blanching response was brought about by means of bi- or uni-lateral stimulation of the eyestalk-stubs of eyestalkless *S. haematocheir*, juv. (Fig. 8). Stimulation was performed either mechanically (pressing with small spherical head of a slender glass rod) or electrically (applying faradic current induced by the Harvard Inductorium connected to a 2 volts source; employing the bi- or uni-polar method). A comparison of time relation curves of reactions of the black chromatophores to respective treatments with that obtained as the result of amputation of eyestalks, indicated that all the reactions occurred under similar circumstances in the immediate surrounding of the chromatophores. In other words, it may be concluded that stimulation of the optic tract activates an incretory mechanism of the

brain or other ganglia to deliver the hormone responsible for pigment concentration in the black chromatophores.

Regarding such possible nervous control of hormone secretion by the nervous tissues, an attempt was made to inquire into the effect of acetylcholine, pilocarpine and atropine. These were injected in doses ranging from 2.5 γ to 50 γ, but none of them was responsible for any positive effect (*i.e.*, pigment concentration in the black chromatophores).

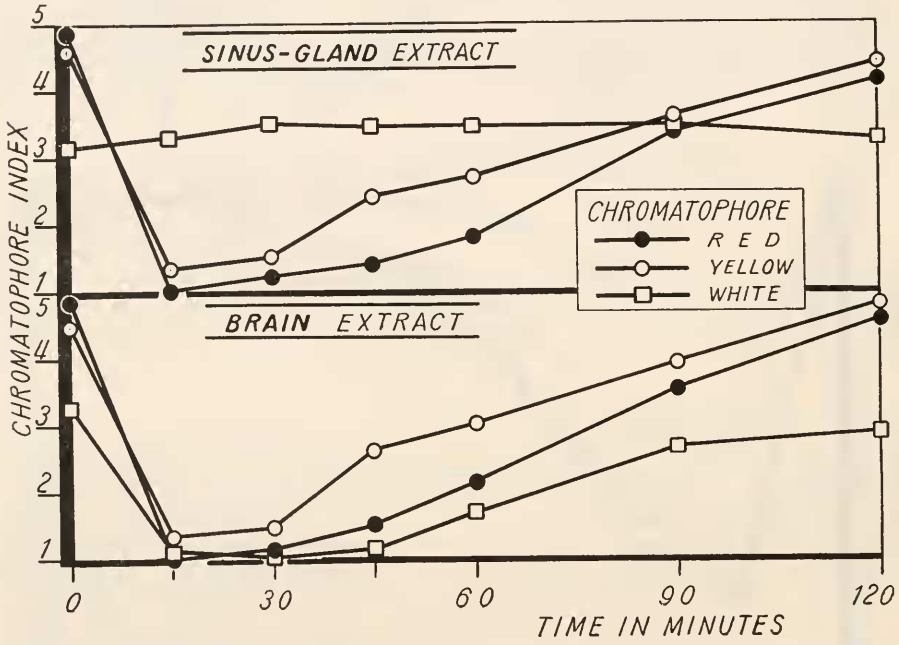

FIGURE 9. Curves showing time relations of responses of red, yellow, and white chromatophores of eyestalkless *Paratya compressa* to injection of active extracts from adult *S. intermedia*. Concentration was 0.5 T.E. per 0.01 cc. in respective extracts.

Mutual Independence of Peripheral Actions of the Two Kinds of Hormones

Assuming on the basis of the foregoing results that the sinus gland contains a hormone affecting the red and the vermilion chromatophores of *S. haematocheir*, juv., while the brain, besides containing a principle like that of the sinus-gland, also furnishes a hormone acting on the black and white cells, double injections of these extracts were tried for the purpose of learning something about any possible interference between the peripheral actions of the two hormones.

At first one of the extracts was injected, and at the end of 5 minutes the second injection of the other extract followed. In another series of experiments mixtures in various proportions of the two extracts were applied to isolated carapace fragments maintained *in vitro*.

In no case was there any significant antagonism between the two extracts. The response of the black chromatophores was always dependent upon the concentration of the brain extract, being by no means significantly affected by the co-existing

sinus-gland extract. The magnitude of response of the red and the vermilion cells was variously amplified in every instance over the range determined by the sinus-gland extract alone. This may be explained by possible summation of the effects of the hormone responsible for the red and vermilion cells present in both of the extracts applied, on the assumption that the hormone originating in the brain is identical with the sinus-gland hormone at least as to the peripheral activity.

Chromatophorotropic Activities of Extracts from Sesarma as Tested upon Paratya, Ocypoda and Megaligia

In order to obtain further information concerning the possible differentiation into two kinds of chromatophorotropic hormones in Sesarma, the activities of the effective extracts from adult forms were assayed upon the chromatophores of eyestalkless Paratya, eyestalkless Ocypoda, and "blinded" and "white-adapted" Megaligia. Results of injection experiments are summarized in Table II.

The table shows that the red and the yellow chromatophores of Paratya were influenced by the hormone widely distributed in the sinus gland as well as in all of the principal nervous organs of adult Sesarma, while the white cells were under the exclusive control of another kind of hormone originating in the brain and Medulla terminalis (cf. Fig. 9 also). However, the chromatophores of Ocypoda and Megaligia did not exhibit qualitatively different responses to the administration of different kinds of hormones of Sesarma.

Discussion

So far as the principal results of the present study are concerned, the endocrine systems of *Sesarma intermedia, S. haematocheir* and *S. dehaani* are to be classified into two categories on the basis of the distribution of the possible sources of chromatophorotropic hormones. One is represented by the sources of a hormone or a group of hormones which is responsible for correlated pigment concentration in the red and the vermilion chromatophores of *Sesarma haematocheir,* juv., and in the red and the yellow chromatophores of *Paratya compressa.* Such a kind of hormone is characteristic of the sinus gland, the universally admitted significant source of chromatophorotropic hormone, but, in Sesarma, a similar hormone is originating in all the principal nervous organs too, which fact is borne out by the wide distribution in the nervous systems of the crabs of certain incretory elements assumed to produce the active principle comparable to that of the sinus gland. Assuming tentatively the identity of the hormone originating in the nervous tissues with that in the sinus gland, I should like to propose the name S hormone (hormone characteristic of sinus gland) which comprises both the sinus-gland hormone and the one from the nervous tissues in the present cases.

The other endocrine system is represented by sources of a hormone responsible for correlated pigmentary reactions in the black (pigment concentration) and the white (pigment dispersion) chromatophores of *S. haematocheir,* juv. and pigment concentration in the white chromatophores of Paratya. In contrast to the wide distribution of the possible sources of S hormone, the range of distribution of the possible sources of this second kind of hormone is limited to particular portions of the nervous system, *i.e.*, brain and Medulla terminalis in adult forms, and brain, Medulla terminalis, thoracic and commissural ganglia in juvenile forms. In view

of the restriction of its possible source in the nervous tissues, I wish to designate such a hormone under the name N hormone (hormone essentially originating in the nervous tissues).

That a type of hormone, whose effect is qualitatively identical with the sinus-gland hormone, arises from the nervous tissues was already implied by Brown (1933), Hosoi (1934), Enami (1943), etc., who demonstrated certain measures of chromatophorotropic activity in extracts of various parts of the central nervous systems of different crustaceans. With *Uca dubia,* Enami proved that production of such a hormone in the brain and the thoracic ganglion is independent of the activity of the sinus gland, and, as already demonstrated, such is also the case with the present Sesarma.

Brown and Ederstrom (1940) offered the first concrete evidence with Crago of the occurrence in certain portions of the nervous system of a kind of hormone which is different from the sinus-gland hormone in its qualitative effect. A hormone which has thereafter been dealt with as CDH (Crago-darkening hormone) was at first detected in maximum concentration in extracts of the commissural ganglion, but later works by Brown and Wulff (1941) and Brown (1946) determined the site of essential source of this hormone to be the tritocerebral commissure. This CDH is said to be responsible for pigment dispersion in the black chromatophores distributed at the telson and uropods of Crago. Following the discovery of CDH, Brown and his associates (Brown and Wulff, 1941; Brown and Klotz, 1947) were successful in detecting and separating a second chromatophorotropic hormone (CBLH, Crago body-lightening hormone) originating likewise in the tritocerebral commissure, which is responsible for pigment concentration in the black chromatophores of the body except the telson and uropods. Antagonism was proved between these two kinds of hormones; in the absence of CBLH, CDH is effective in inducing pigment dispersion of the black cells over the whole body surface. Using Crago as test animal, Brown and Saigh (1946) examined the comparative distribution in the nervous systems of CDH and CBLH in 13 species representing Isopoda, Natantia, Astacura, Anomura and Brachyura, obtaining positive results with most of the animals. According to these authors, CBLH has in most cases wider distribution in the nervous systems as compared with CDH, which is rather restricted to certain particular portions of the nervous organs.

From the point of view of qualitative effect, what is designated as CBLH appears to be identical with the sinus-gland hormone, whose activity upon Crago black chromatophores has been determined since the pioneering work by Koller (1928). The identity of these hormones seems to be further substantiated by the work of Brown and Ederstrom (1940) who remarked that CDH and the sinus-gland hormone were mutually antagonistic. Further, the work by Brown and Klotz (1947) demonstrated that CBLH was considerably soluble in organic solvents, such as ethyl, methyl and isopropyl alcohols, in contrast to CDH, which had no significant solubility in these solvents, which fact appears to point out the similarity in chemical properties of CBLH to the sinus-gland hormone which has been demonstrated to be also considerably soluble in alcoholic solvents (Carlson, 1936; Abramowitz and Abramowitz, 1938; Abramowitz, 1940; Brown and Scudamore, 1940).

Such a consideration seems to permit a tentative generalization stating that CBLH together with the sinus-gland hormone may be dealt with under the same

category of chromatophorotropic hormones, which apparently corresponds to the present S hormone of Sesarma. Such a discussion appears to be premature in the present report, but it is tenable in that certain histological evidence proving the identity of secretory behaviors of the sinus gland and incretory elements found in the nervous systems was obtained with Sesarma, which will be published in a subsequent paper.

Assuming now that the nervous systems of crustaceans are generally charged with two kinds of chromatophorotropic hormones, as already postulated and proved by Brown, the present N hormone in Sesarma might be conceded to correspond to the said CDH, notwithstanding that CDH was reported to be absent in several brachyurans, such as *Libinia* sp., *Cancer irroratus, Carcinides maenas, Ovalipes ocellatus* and *Uca pugilator* (Brown and Saigh, 1946). As stated above, no significant antagonism was detected between S and N hormones when assayed upon the chromatophores of *S. haematocheir,* juv., but an inquiry into mutual interaction of these hormones is desirable upon the black chromatophores of Crago, before implied disparity of N hormone from CDH is taken into consideration. Brown and Saigh reported a differential distribution of CDH in the nervous systems of the crustaceans examined. In Sesarma, the distribution of N hormone in the nervous organs differs between the adult and the juvenile forms, being restricted in the former.

Hitherto no definite information was published as regards the production of chromatophorotropic hormones in the nervous tissues included in the eyestalk. Present investigation disclosed the universal occurrence of S hormone in the ganglia of the eyestalk of Sesarma and the restricted distribution of N hormone in the Medulla terminalis. On the basis of such knowledge, the data presented by Brown (1940) indicating the presence of considerable activity in the rest of the eyestalk tissues outside the sinus gland are to be attributed to the possible presence of S hormone in the ganglionic tissues in the eyestalks of crustaceans examined, *viz.,* Uca, Carcinus, Pagurus, Palaemonetes, Libinia, Callinectes and Crago. Further, Brown (1940) reported that the white chromatophores of Palaemonetes were, unlike the red ones, not significantly susceptible to the sinus-gland extract, which, according to an earlier paper by the same author (1935), were reported to be affected by the extract of the whole eyestalk. This fact appears to be explained on the assumption that N hormone is included in certain parts of ganglionic tissues in the eyestalk of the shrimp.

Thus evidence has been accumulating toward confirmation of the presence of two kinds of chromatophorotropic hormones in crustaceans, and it is to be added here that in Cambarus too McVay (1942) was cited by Brown (1944) as being successful in separating two hormones originating in the central nervous system.

Summary

1. Chromatophorotropic hormones in three species of Sesarma, *viz., S. intermedia, S. haematocheir* and *S. dehaani,* are differentiated into two broad categories of S and N hormones.

2. S hormone is responsible for pigment concentration in the red and the vermilion chromatophores of juvenile forms of *S. haematocheir,* and also for pigment concentration in the red and the yellow cells of *Paratya compressa,* while N hormone is responsible for pigment concentration in the black chromatophores and pigment

dispersion in the white ones of *S. haematocheir*, juv., and also for pigment concentration in the white cells of Paratya.

3. S hormone is distributed not only in the sinus gland but in all the principal nervous organs in both the adults and the juvenile forms of Sesarma. Occurrence of S hormone in the nervous tissues is independent of the activity of the sinus gland, suggesting the presence of a certain endocrine organization in the nervous system of the crabs.

4. N hormone is restricted to the brain and the Medulla terminalis in the adults, being widely distributed in the brain, the Medulla terminalis, the thoracic and the commissural ganglia in the juvenile forms. Production of the hormone in these ganglionic tissues is also unaffected by the activity of the sinus gland. Stimulation of the optic tract is responsible for secretion of N hormone.

5. Antagonism between S and N hormones could not be detected through the assay with the chromatophores of *S. haematocheir*, juv.

6. The discussion is especially concerned with recent information on the sources and activities of chromatophorotropic hormones originating in the nervous tissues in other species of crustaceans.

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